Exhibit 10

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Review

Strategies for the identification, control and determination of genotoxic impurities in drug substances: A pharmaceutical industry perspective

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ABSTRACT

Regulations alarmed the control of genotoxic impurities in drug substances at lower level based on the threshold of toxicological concern and daily dose. This review explores the details of various regulations and guidances, toxicology assessment, identification of structural alerts, synthetic origins, different synthetic approaches for elimination or control, various analytical determination strategies and pharmaceutical industry concern towards genotoxic impurities.

Contents

1. Introduction

Compounds that can induce genetic mutations, chromosomal breaks, and/or chromosomal rearrangements are considered as genotoxic impurities (GTIs) and have the potential to cause cancer

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in humans [1,2]. ICH and EMEA guidelines provide the limits for impurities in drug substances and drug products [3–6]. These limits are not acceptable for GTIs due to their adverse affects and hence it is necessary to set up limits based on daily dose of the drug substance. Even though this is desirable in quality point of view, it deploys the resources in process development. To overcome this, scientists have to identify GTIs early in process development, develop analytical methods and demonstrate the synthetic process controls. However, the relevant strategies are not readily available to all the drug substance or active pharmaceutical ingredients (APIs) manufacturers. Hence, we have made an effort to present an overview on GTI identification, control and determination strategies in drug substances [7–10].

2. Regulations and guidances

2.1. EMEA guideline

EMEA guideline on the limits of GTIs [11], classifies GTIs into two categories. (a) GTIs with sufficient (experimental) evidence for a threshold related mechanism. These are to be regulated using methods outlined in ICH Q3C(R4) for class 2 solvents [5] and (b) GTIs without sufficient (experimental) evidence for a threshold related mechanism. These are to be controlled 'as low as reasonably practicable' (ALARP principle). Although this approach is acceptable in most instances, mechanistic data sufficient to allow for an assessment of threshold mechanism is lacking. Hence, this guideline proposed the use of 'threshold of toxicological concern (TTC)', that refers to a threshold exposure level to compounds which will not pose a significant risk of carcinogenicity or other toxic effects. A TTC [12] value of 1.5 μg/day intake of GTI is considered to be associated with an acceptable risk. The concentration limit in ppm of GTI permitted in a drug substance is the ratio of TTC in micrograms/day and daily dose in grams/day. The TTC approach benefits consumers, industry and regulators by avoiding unnecessary toxicity testing and safety evaluations. This guideline summarizes its recommendations in the form of a decision tree in which the preferred option is to eliminate GTIs, second preference is to apply ALARP principle and the final alternative is the TTC approach. EMEA also released "Question and Answer" document [13] by clarifying questions arouse in its original guidance [11].

2.2. PhRMA approach

The Pharmaceutical Research and Manufacturing Association (PhRMA) derived a procedure for testing, classification, qualification and toxicological risk assessment of GTIs [14]. It provided some structurally alerting functional groups (structural alerts or alerting structures) that are known to be involved in reactions with DNA. These were categorized into three groups. Group 1: aromatic groups e.g. N-hydroxyaryls, N-acylated aminoaryls, aza-aryl N-oxides, aminoaryls and alkylated aminoaryls, purines or pyrimidines, intercalators, PNAs or PNAHs, Group 2: alkyl and aryl groups e.g. aldehydes, N-methylols, N-nitrosamines, nitro compounds, carbamates (urethanes), epoxides, aziridines, propiolactones, propiosultones, N or S mustards (beta haloethyl), hydrazines and azo compounds, Group 3: hetero aromatic groups e.g. Michael-reactive acceptors, alkyl esters of phosphonates or sulphonates, haloalkenes, primary halides (alkyl and aryl-CH_2). PhRMA categorized impurities into five classes. Class 1: impurities known to be both genotoxic (mutagenic) and carcinogenic. These impurities represent the most serious risk and the default preference is to eliminate them by modifying the process. If this is not possible, TTC concept must be employed as a last option. Class 2: impurities known to be genotoxic (mutagenic) but with unknown carcinogenic potential. These impurities are to be controlled using TTC principles. Class 3: impurities containing alerting structures, unrelated to the structure of the API and of unknown genotoxic (mutagenic) potential. This group includes impurities with functional moieties that can be linked to genotoxicity based on structure. Class 4: impurities containing alerting structures which are related to the API. This group includes impurities that contain an alerting functional moiety that is shared with the parent structure. Class 5: impurities with no alerting structures or sufficient evidence for absence of genotoxicity. These are to be treated as normal impurities and controlled according to the ICH guidelines. If class 3 or 4 compounds are genotoxic, or not tested, they are moved into class 2 and if these are nongenotoxic, they are considered as class 5. Several approaches can be found in this article [14].

2.3. USFDA guidance

USFDA released draft guidance [15] to address GTI issues. This guidance describes a variety of ways to characterize and reduce the potential lifetime cancer risk associated with patient exposure to genotoxic and carcinogenic impurities. The recommended approaches include (a) prevention of genotoxic and carcinogenic impurity formation, (b) reduction of genotoxic and carcinogenic impurity levels (allowing a maximum daily exposure target of 1.5 μg/day), (c) additional characterization of genotoxic and carcinogenic risk and (d) considerations for flexibility in approach to better support appropriate impurity specifications.

2.4. European pharmacopoeial guidance

European pharmacopoeia requires a pragmatic approach on GTIs when elaborating or revising monographs. It says that the products that receive a marketing authorization after the issuance of the EMEA guideline [11] have to be evaluated for the presence of GTIs and this should be the basis for a new monograph [16].

2.5. Guidance for oncology products

TTC limits may be liberalized for GTIs for oncology products [17]. The USFDA draft guidance [15] states, 'a TTC value higher than 1.5 μg per day may be acceptable in situations where the anticipated human exposure will be short term, for the treatment of life threatening conditions, when life expectancy is less than 5 years, or where the impurity is a known substance and human exposure will be much greater from other sources'. The ICH S9 guideline on nonclinical evaluation for anticancer pharmaceuticals [18] also states, 'for genotoxic impurities, several approaches have been used to set limits based on increase in lifetime risk of cancer. Such limits are not appropriate for pharmaceuticals intended to treat patients with advanced cancer and justifications should be considered to set higher limits'.

3. Toxicology assessment

Toxicology assessment [19,20] is to be done by pharma scientists and toxicologists to identify GTIs and their entry into the synthetic process, to search for the opportunities for their removal and provide limits that are consistent with safety and regulatory expectations. This evaluation can be done via literature review or computational toxicology assessment. Ashby and Tennant [21,22] introduced the concept of identification of structural alerts for genotoxic activity based on their correlations between electrophilicity and DNA reactivity as assessed by Ames-testing data [23]. In literature, articles containing several structural alerts were published [21–25]. The commonly used software includes MDL–QSAR [26], MC4PC [27] and DEREK

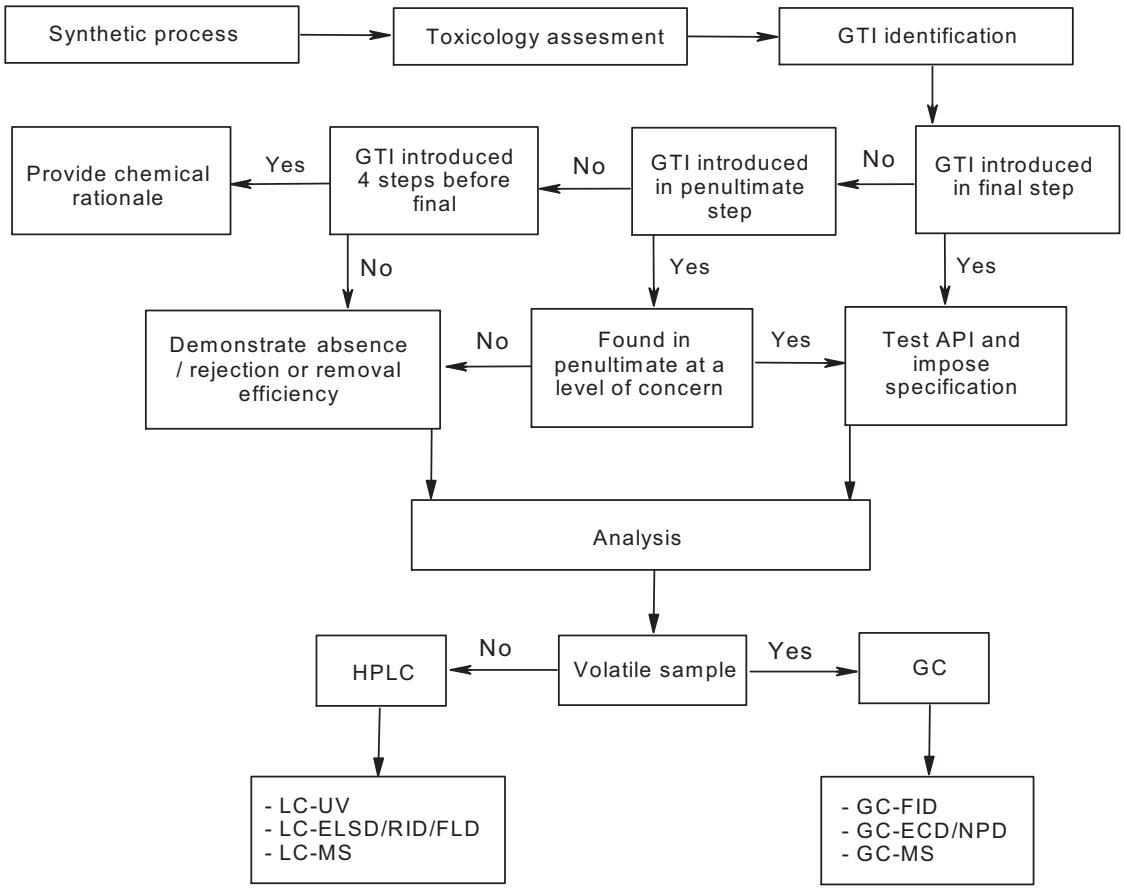

Fig. 1. Flow diagram for the identification, control and determination of GTIs in drug substances.

for Windows [28]. However, due to the uncertain relevance of structural alerts, regulatory action should not be based solely on the presence of a particular functional group. The accuracy for predicted genotoxicity should be evaluated case by case based on the available literature and genotoxicity test results.

4. Synthetic approaches

4.1. Identification of GTIs

In a synthetic process, a GTI may be introduced as a starting material, reagent, intermediate, catalyst, by-product, isomer or degradant. Pierson et al. [29] proposed a simulated synthetic route representing the way of entry of GTIs. Alkyl halides [30] used as reagents in synthesis are genotoxins. These are also generated during chemical synthesis when a salt counter ion (e.g. hydrogen halide) of a drug substance reacts with alcohols. Methane sulfonic acid (mesylate), benzene sulfonic acid (besylate) and p-toluenesulfonic acid (tosylate) are commonly used as counter ions to form API salts [31–33]. Interactions of these acids with residual alcohols may lead to the generation of GTIs. For example alkyl methane sulphonates, alkyl benzene sulphonates, alkyl p-toluene sulphonates may associate with imatinib mesylate [7], amlodipine besylate [8] and denagliptin tosylate, respectively. Esomeprazole magnesium is obtained from the resolution of racemic omeprazole magnesium using camphor sulfonyl chloride, if alcohols are used in the synthetic process, alkyl camphor sulphonates may associate with the drug substance [10]. Alkyl esters of sulphate [9] are also genotoxins (e.g. dimethyl sulphate is used in the synthesis of pantoprazole sodium). Epoxides and hydroperoxides [34] have genotoxicity. Isomers of some drug impurities are found to be genotoxic (e.g. EE isomer of terbinafine impurity) [35]. In addition to the usage of genotoxic reagents, alerting degradants may also form in the process. Raillard et al. [36] presented the formation of GTIs in drug substances during degradation. Aldehydes, α, β- unsaturated carbonyls, aromatic amines, hydroxylamine and its derived esters, epoxides and polyaromatic hydrocarbons account for structural alerts in degradants. An alerting degradant form in two main ways, (a) parent drug that already contains a structural alert. This is again of two types (i) degradant with same alerting structure as that of drug is formed e.g. oxybuprocaine with a structural alert for aromatic amines forms an acid degradant having the same structural alert for aromatic amines via hydrolysis, (ii) degradant with a different alerting structure than parent drug is formed, e.g. acetaminophen containing a structural alert for N-acylated aminoaryls forms p-aminophenol having a new structural alert viz aromatic amine, (b) parent drug with no alerting structure forms a degradant containing an alerting structure, e.g. propofol, which lacks a structural alert, degrades via oxidation to a dimeric degradation product containing several conjugated unsaturated carbonyl systems, which are structural alerts for mutagenicity.

4.2. Synthetic strategies in eliminating GTIs

It is always advisable for synthetic scientists to explore possible opportunities to avoid the use and generation of GTIs in the synthetic process. It may not always be practical, but changing synthetic route during development particularly as processes are scaled up is useful to control or reduce GTIs. The main strategy [35] is the redesigning the synthetic process to avoid GTIs e.g. synthesis of denagliptin tosylate [37]. In many cases, GTIs have been success-

fully reduced below the TTC, simply by either altering appropriate reaction conditions like changing proportions of reaction components, interchanging of the reaction addition modes, changing the key starting materials, starting with different intermediates, changing functional groups with structural alerts, etc. or trying with different mechanisms. This can often be achieved without significant loss of yield. GTIs can also be reduced in workup stages like crystallization, isolation, washings and drying [38].

4.3. Synthetic justifications

GTIs can mainly be addressed as a function of their entry into the synthetic route [29]. Demonstrating synthetic process capability in their removal, routine GTI testing can be avoided. The justification strategies are (a) GTI is introduced in the final step: a specification should be applied for GTI on the basis of the toxicology assessment. If data is generated to show that a GTI introduced in the last step is not actually present or efficiently rejected, it may be possible to omit a specification, (b) GTI is introduced in the penultimate step: if GTI is shown to be below toxicological limit in the penultimate step, no testing is required for API. If the GTI is present at the level of concern in the penultimate step, a specification is to be applied to the API to verify adequate removal in the last step, (c) GTI is introduced four steps before final step: sufficient data should be established to show the rejection of GTI in any of the subsequent intermediate steps through spiking studies and no special testing or control is required for the scale-up of intermediates used in the production of API. If the removal is not possible in the intermediate step, a specification limit for the API is needed and (d) GTI is introduced greater than four steps before final step: chemical rationale can be provided that the GTI carryover to the API is negligible. This can be based on (i) reactivity of the GTI in subsequent step, (ii) number of purification steps that will encounter, (iii) its solubility in the extraction solvents and (iv) its solubility in mother liquor while filtration.

5. Analytical challenges

5.1. Analytical strategies

The final API determination methods are not suitable for GTIs determination since their quantitation limit (QL) is generally 100 ppm (0.01%). Hence, highly sensitive methods are required to determine GTIs because of their lower quantitation limits. In the analytical method point of view, the actual QL could be much more sensitive than the GTI concentration limit. In addition to this, certain drug substances may generate GTIs via degradation [36] or storage and are to be separated from process impurities to have specificity in the analysis. Besides sensitivity and specificity, the other challenges in GTIs determination include (a) diverse structural types of GTIs which require the application of various analytical techniques, (b) GTIs without structural features are not amicable to common analytical detectors, (c) chemically reactive or unstable GTIs lead to low recovery and poor sensitivity and requires special handling techniques, (d) interference of sample matrix [39] resulted by enhanced test concentration of API to achieve lower detection limits. Sample solubility is also one of the major issue, e.g., in saquinavir mesylate, mesylate forms methyl methanesulphonate (MMS) with methanol. But the drug substance is soluble only in methanol (diluent) among the various common solvents available. In this case, the diluent forms MMS, which is actually to be determined. However, extraction procedures are useful in these cases. To face these challenges various analytical strategies, sample preparation methodologies, chromatographic separation tools and detectors are to be explored. The method should serve purpose and level of testing. In addition to sensitivity and specificity requirements, parameters like detection limit, quantitation limit, linearity and range, accuracy (recovery) and solution stability are to be established as per ICH Q2 (R1) validation guidelines [40]. However, the extent of validation depends on the purpose of the study.

5.2. Selection of analytical technique

Liu et al. reported method development strategy, advances and control of GTIs [41–43]. The analytical technique selection can be done by dividing GTIs into two groups based on their volatility. HPLC with UV detection shall be selected for non-volatile GTIs in general as first choice due to their simplicity and availability [8]. However, often they may not offer sufficient sensitivity for certain GTIs in lower level analysis. If GTIs offer insufficient UV response, ultra performance/fast liquid chromatography (UPLC or UFLC) can be used due to their enhanced UV detector sensitivity. When GTIs are in lack of chromophores, evaporative light scattering detector (ELSD) is the alternate choice. But, this detector is limited in sensitivity and dynamic range. Refractive index detector (RID) and fluorescence detector (FLD) are the other alternate detectors used in HPLC. Since, lower QL establishment is challenging, hyphenation of HPLC or UPLC with mass detector (MS or MS/MS) will significantly improve the method sensitivity and makes the methods more rapid [32,44]. These detectors are selective, minimize issues caused by interferences in the sample matrix and thus improve data quality. However, these instruments are expensive, differ from vendor to vendor and thus transferring a method between development and receiving laboratories, if contain instruments from different vendors, require optimization of multiple instrumental parameters [41]. Volatile GTIs can be quantitated by GC with flame ionization detector (FID) [45,46] as standard first attempt in direct and headspace injection modes depending on properties of GTIs and sample matrices. Electron capture detector (ECD) can be used when GTIs consist of halogens. Nitrogen–phosphorus detector (NPD), offer an additional tool for GTIs containing nitrogen and/or phosphorus atoms. However, the applications of these two detectors are limited. GC–MS offers the most sensitive and selective detection, reduced background noise and less prone to interferences for low level analysis of GTIs [7,47]. If GTIs are labile, do not possess chromophores and have reactive functional groups, they can be derivatized to form detectable species (e.g. hydrazine derivatizes with benzaldehyde to form 1,2-dibenzylidenehydrazine) [44,48]. Derivatization reagent is selected based on the functional groups in the analyte. Derivatization helps in stabilization, incorporation of a unique structural moiety, enhancing fluorescence, ionization for mass detection, volatization for GC, etc.

5.3. Analytical justifications

Good scientific judgment is needed when decisions are being taken, depending on the specific situation like (a) when it is difficult to develop methods with QL less than 1 ppm. For instance, in levetiracetam, chloro butyryl chloride is to be quantitated at 0.5 ppm level. In such a case, GTI may be quantitated at a level 'as low as possible'. A justification may be given by explaining analytical capability, (b) being reactive in nature, many GTIs are unstable for direct analysis, e.g. acid chlorides (valeryl chloride) converts to acids (valeric acid) in acidic diluents. In such cases, justification may be provided by analyzing them as their derivatives and (c) when multiple GTIs are structurally similar in nature (e.g. polyaromatic hydrocarbons and polymer oligomers), chromatographic separation or individual control of each GTI may be more difficult to achieve. In such cases, it may be proposed that the group collectively meets the exposure limits as if it were one sin-

gle GTI. However, it cannot be considered that all these GTIs have the same degree of genotoxicity and/or analytical response factor. The flow diagram representing the GTI identification, control and determination in drug substances is shown in Fig. 1.

6. Pharmaceutical industry concern

Pharmaceutical industry has great concern towards GTIs due to their adverse effects. Hence, a GTI free API should provide the following. (a) Synthetic scheme showing the origins, point of entry and removal of GTIs, (b) toxicology assessment, (c) a sound scientific appraisal for identified GTIs among all chemicals, impurities, by-products and degradation products of API, (d) justification for the concentration limit with or without a threshold related mechanism, (e) analytical methods for the determination of GTIs and (f) absence study of sulfonic acid esters. Nevertheless, literature evidences that the pharma scientists have identified and controlled GTIs in many drug substances [7–10,30–34,43,49–52].

7. Conclusions

Identification and control of genotoxins in a synthetic process is challenging, owing to its evolving nature and variable points of their entry. Hence, synthetic routes are to be screened for the identification of structural alerts which cause genotoxicity. If GTIs are found, then alternative synthetic routes which can control these impurities should be developed. If this is not technically feasible, then safety limits must be fixed based on TTC concept. These limits generally come into lower levels and need analytical determinations with adequate selectivity and sensitivity. In addition, GTIs need to be addressed on an ongoing basis during drug development. If the route is changed, new intermediate compounds must be assessed. If the acceptable toxicology limit has changed due to the change in daily dose, the capabilities of the process and analytical methods for control at the new level need to be assessed. As a whole, a multidisciplinary collaboration with experts in the areas of toxicology, synthetic and analytical chemistry is required to balance the risk and cost during the development of drug substances. Finally, it is worth to conclude this review with the USFDA statement 'Although marketed medicinal products are required to be safe, safety does not mean zero risk. A safe product is one that has reasonable risks, given the magnitude of the benefits expected and the alternatives available'.

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